Cytoprotection and Preconditioning for Stem Cell Therapy

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1. Introduction

Coronary heart disease is the leading cause of morbidity and mortality worldwide. To date, management of myocardial infarction (MI) has been limited to timely revascularization and drug therapy aimed to restore coronary blood flow and to reduce myocardial workload. When disease progresses to life-threatening end-stage heart failure, heart transplantation is the only effective therapeutic option available. However, its usage is very much restricted by the severe shortage of heart donors and the complications associated with enduring immune suppressive drug treatments (Miniati & Robbins, 2002). Therefore, innovative treatment strategies are clearly needed to improve patient outcomes. Recent advances in stem cell medicine have shed new light on potential MI therapies by exploiting the pluripotency of stem cells for cardiac repair and regeneration. Although immense progress has been made on the choice of cells and optimizing transplantation conditions, these remain critical issues when translating into the clinical setting of MI. In particular, poor survival of transplanted cells in the hostile microenvironment of the ischaemic myocardium and hence lack of significant engraftment in the heart has been a major impediment for achieving an effective stem cell therapy for MI (Pagani et al., 2003). Various cytoprotection strategies have been developed over the past decade to circumvent this limitation and the non-genetic approach of preconditioning has emerged as one of the most promising clinically adaptable strategies to promote stem cell survival and function under various ischaemic conditions. Although genetic enhancement of stem cells has been very successful in pre-clinical studies, the technical complexity and safety concerns (oncogenicity and mutagenesis) associated with this alternative approach have precluded its application in clinical translation (Bonaros et al., 2008; Penn & Mangi, 2008). This review will focus on current pre-clinical development of non-genetic preconditioning approaches to improve the therapeutic potential of stem and progenitor cells for repair of the heart after MI.

2. History of ischaemic and pharmacological preconditioning

The protective phenomenon of preconditioning was first described by Murry et al. in 1986 whereby exposure to brief cycle(s) of sub-lethal ischaemia with intermittent reperfusion, which in itself does not induce injury, render the heart more resistant to subsequent lethal ischaemic insults; this phenomenon was termed ischaemic preconditioning (IPC) (Murry et
al., 1986). Subsequent studies by various laboratories have quickly established IPC as the most powerful and effective means of endogenous protection against ischaemic injury. Although this protective intervention can be easily reproduced in various pre-clinical studies, successful translation into clinical practice has been limited by the safety consideration of needing to manipulate the already injured heart. To circumvent this limitation, effort has been concentrated on clarifying the underlying molecular mechanisms governing the cardioprotective effect of IPC which have lead to the discovery of various pharmacological agents that can directly activate the protective signalling pathways to achieve myocardial protection without ischaemia, an intervention called pharmacological preconditioning. Despite extensive research, the mechanism(s) underlying the protective effect of preconditioning remain to be fully elucidated. It is believed to involve multiple intricate endogenous signalling pathways (Yellon & Downey, 2003; Huffmyer & Raphael, 2009) including agonists of G-protein coupled receptors (adenosine, bradykinin, opioids, etc), growth factors (IGF, TGFβ, VEGF, etc), phosphodiesterase inhibitors, mitochondrial KATP channel openers, cytokines (TNFα, IL-1β, IL-6, etc), nitric oxide (NO), and others. Some of these have been promoted into the clinical arena, for example adenosine in AMISTAD I (Mahaffey et al., 1999) and II (Ross et al., 2005) trials. In general, non-genetic preconditioning strategies employed by current pre-clinical studies to improve survival and function of stem and progenitor cells can be categorized into ischaemic/hypoxic and pharmacological preconditioning.

3. Ex vivo ischaemic and hypoxic preconditioning of stem cells

The ischaemic conditions used to simulate IPC in vitro are quite diverse. The majority of studies have experimented with hypoxia or anoxia alone, termed hypoxic preconditioning (HPC), while others include nutrient deprivation. In some studies, hydrogen peroxide (H2O2) was used to simulate the ischaemic condition of oxidative stress (Li et al., 2009; Sharma et al., 2008). Furthermore, different HPC protocols, from the classical multiple cycles of brief hypoxia with intermittent reoxygenation to a single long-term exposure to hypoxia, have been employed to demonstrate the cytoprotective effect of HPC on stem and progenitor cells in vitro. It is also interesting to note that subjecting stem and progenitor cells to heat shock, as a form of sublethal cell stress, also capable of promoting their survival and in vivo engraftment (Laflamme et al., 2005; Maurel et al., 2005; Suzuki et al., 2000). Despite these differences and the lack of an optimal protocol definition, the beneficial effects of HPC on stem and progenitor cell function were unequivocally demonstrated in all these studies and involved multiple signalling molecules (Table 1) (Figure 1).

3.1 Survival

The poor survival and retention of transplanted stem and progenitor cells has driven the investigation towards effective cytoprotective strategies which aim to enhance their survival in the ischaemic environment. The extent of retention of the delivered cells was documented to be rather low with recent studies suggesting that more than 90% are lost partly because of necrosis and apoptosis in the ischaemic myocardium following their delivery by intramyocardial, retrograde transvenous, intracoronary or systemic routes (Terrovitis et al., 2010; Aicher et al., 2003; Goussetis et al., 2006). One means of overcoming this limitation would be to increase the survival of transplanted cells, thus avoiding the impractical and costly alternative of delivering large excesses of stem cells into the injured myocardium.
Cytoprotection and Preconditioning for Stem Cells Therapy

Fig. 1. Mechanisms underlying the cytoprotective effect of hypoxic preconditioning in stem and progenitor cells.

HPC by sublethal hypoxia has been shown to enhance the survival of stem and progenitor cells isolated from various species including humans in vitro (Table 1). Similarly, the cytoprotective effect of HPC was demonstrated in a number of in vivo studies using clinically relevant ischaemic models (Table 2). In these studies, ex vivo HPC rendered stem and progenitor cells more resilient to cell death when transplanted into the infarcted myocardium (He et al., 2009; Hu et al., 2008; Tang et al., 2009; Uemura et al., 2006), ischaemic limb (Akita et al., 2003; Kubo et al., 2008; Li et al., 2002; Rosova et al., 2008) or ischaemic brain (Theus et al., 2008), and this finding was significantly correlated with improved functional recovery of the ischaemic tissues. For instance, transplantation of hypoxic preconditioned mesenchymal stem cells (MSCs) into the ischaemic myocardium showed enhanced therapeutic benefits in terms of infarct size reduction, increased angiogenesis, improved ventricular function and less adverse cardiac remodelling (Hu et al., 2008). These beneficial effects of hypoxic preconditioned stem cells are attributed to enhanced pleiotropic paracrine activities instead of transdifferentiation and cell fusion, which occurred at insignificantly low frequency. Convincing evidence in support of the paracrine paradigm were provided by in vitro studies with conditioned media, where media from hypoxia-conditioned MSCs was shown to be cytoprotective in cultured human aortic endothelial cells (Hung et al., 2007a) and primary rat cardiomyocytes (Gnecchi et al., 2006) subjected to hypoxic injury.

Mechanistic evaluations of the cytoprotective effect of HPC in stem and progenitor cells have implicated the up-regulation of a diverse array of soluble survival proteins such as growth factor VEGF (Akita et al., 2003; Potier et al., 2007), anti-apoptotic proteins Bcl-2 and Bcl-xL (Francis & Wei, 2010; Hu et al., 2008; Theus et al., 2008; Wang et al., 2008a), antioxidants heme oxygenase-1, hexokinase-2, catalase and superoxide dismutase (Kubo et
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<tr>
<td>MSCs (mouse)</td>
<td>&lt;0.1% O₂, 4 h</td>
<td>↑ survival of cocultured adult CMs</td>
<td>Akt; VEGF; SDF-1; eNOS</td>
<td>Uemura et al., 2006</td>
</tr>
<tr>
<td>MSCs (mouse)</td>
<td>0.5% O₂, 24 h</td>
<td>↑ survival</td>
<td>HIF-1α; Ang-1; VEGF/Flik1; Bcl-2; Bcl-xL; p105; EPO; NFκB</td>
<td>Hu et al., 2008</td>
</tr>
<tr>
<td>MSCs (mouse)</td>
<td>0.5% O₂, 24 h</td>
<td>↑ survival</td>
<td>HIF-1α; EPO; Bcl-2; Bcl-xL</td>
<td>Theus et al., 2008</td>
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<td>MSCs (mouse)</td>
<td>1% O₂, 36 h</td>
<td>Conditioned media ↑ cell migration</td>
<td>Wnt4</td>
<td>Leroux et al., 2010</td>
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<td>MSCs (mouse)</td>
<td>3% O₂, 3-24 h</td>
<td>↑ survival; ↑ cell migration; ↑ cell adhesion</td>
<td>Akt; HIF-1α; CXCR4; CXCR7</td>
<td>Liu et al., 2010</td>
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<td>MSCs (rat)</td>
<td>&lt;0.1% O₂ &amp; serum-free, 3 h</td>
<td>↑ survival of cocultured neonatal CMs</td>
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<td>He et al., 2009</td>
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<td>MSCs (rat)</td>
<td>0.5% O₂, 12 h</td>
<td>Conditioned media ↑ adult CMs survival</td>
<td>VEGF; HGF; bFGF; Thymosin β4</td>
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<td>MSCs (rat)</td>
<td>0.5% O₂, 24 h</td>
<td>↑ survival</td>
<td>Akt; VEGF; HIF-1α</td>
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<td>MSCs (rat)</td>
<td>1% O₂, 24 h</td>
<td>↑ survival</td>
<td>Catalase, Mn-SOD, p38MAPK, Bcl-2</td>
<td>Peterson et al., 2011</td>
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<td>MSCs (rat)</td>
<td>2% O₂, 4-48 h</td>
<td>↑ angiogenic factors secretion</td>
<td>VEGF/Flik1; VE-cadherin</td>
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<td>MSCs (rat)</td>
<td>8% O₂, 10-30 min</td>
<td>↑ survival</td>
<td>Akt; ERK1/2; Bcl-2; Bax; VEGF</td>
<td>Wang et al., 2008a</td>
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<tr>
<td>MSCs (rat)</td>
<td>&lt;0.1% O₂, 10/30 min x 1-3</td>
<td>↑ survival</td>
<td>Akt; ERK1/2; Bcl-xL; HIF-1α; miR-210</td>
<td>Kim et al., 2009</td>
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<td>MSCs (human)</td>
<td>1% O₂, 22 h</td>
<td>↑ cell migration</td>
<td>HIF-1α; CX3CR1; CXCR4</td>
<td>Hung et al., 2007b</td>
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<td>MSCs (human)</td>
<td>1% O₂, 2 d</td>
<td>↑ angiogenic factors secretion ↓ osteogenic diff.</td>
<td>VEGF</td>
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<td>MSCs (human)</td>
<td>1% O₂ &amp; serum-free, 2 d</td>
<td>Conditioned media ↑ endothelial cell survival &amp; angiogenesis</td>
<td>Akt; IL-6; eNOS</td>
<td>Hung et al., 2007a</td>
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<td>MSCs (human)</td>
<td>2% O₂, 3 d</td>
<td>Restore hypoxia-induced ↓ of osteogenic diff.</td>
<td>-</td>
<td>Volkmer et al., 2010</td>
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<td>MSCs (human)</td>
<td>1-3% O₂, 16 h</td>
<td>↑ cell migration</td>
<td>Akt; HGF/cMet</td>
<td>Rosova et al., 2008</td>
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<td>PB-MNCs (mouse)</td>
<td>2% O₂, 24 h</td>
<td>↑ survival</td>
<td>HO-1, autocrine motility factor, hexokinase-2</td>
<td>Kubo et al., 2008</td>
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<td>PB-MNCs (mouse)</td>
<td>2% O₂, 24 h</td>
<td>↑ cell migration</td>
<td>VEGF; NOS</td>
<td>Li et al., 2005</td>
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<td>PB-MNCs (human)</td>
<td>P&lt;sub&gt;O2&lt;/sub&gt; of 35mmHg, 7 d</td>
<td>↑ endothelial diff.; ↑ cell migration</td>
<td>VEGF; KDR tyrosine kinase</td>
<td>Akita et al., 2003</td>
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<td>ESNPCs (mouse)</td>
<td>1% O₂, 4-12 h + 24 h Reoxy.</td>
<td>↑ survival; ↑ neuronal diff.</td>
<td>HIF-1α; EPO; Bcl-2</td>
<td>Theus et al., 2008</td>
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<tr>
<td>ESNPCs (human)</td>
<td>0.1% O₂, 12 h + 0-5 d Reoxy.</td>
<td>↑ survival; ↑ neuronal diff.</td>
<td>HIF-1α; HIF-2α; EPO; VEGF; Bcl-2; Bax; Akt</td>
<td>Francis &amp; Wei, 2010</td>
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<tr>
<td>ASCs (human)</td>
<td>1% O₂, 24 h + 24 h Reoxy.</td>
<td>↑ survival of co-cultured NSCs</td>
<td>-</td>
<td>Oh et al., 2010</td>
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<tr>
<td>CLK (mouse)</td>
<td>0.1% O₂, 4-24 h</td>
<td>↑ cell migration</td>
<td>HIF-1α; SDF-1/CXCR4</td>
<td>Tang et al., 2009</td>
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<td>Bone marrow CD133+ (human)</td>
<td>1.5% O₂, 24 h + 2 d Reoxy.</td>
<td>↑ endothelial diff.; ↑ angiogenic-related genes</td>
<td>-</td>
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<td>NSCs (mouse)</td>
<td>0.5% O₂, 3 h</td>
<td>↑ functional engraftment</td>
<td>Connexin-43</td>
<td>Jaderstad et al., 2010</td>
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**Hydrogen peroxide**

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<th>Effects</th>
<th>Contributing Factors</th>
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<td>MSCs (rat)</td>
<td>20 μM H₂O₂, 24 h</td>
<td>↑ survival</td>
<td>SDF-1/CXCR4; ERK1/2</td>
<td>Li et al., 2009</td>
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<td>NPCs (mouse)</td>
<td>0.05-0.5 μM H₂O₂, 24 h</td>
<td>↑ survival</td>
<td>-</td>
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**Heat shock**

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<th>Stem Cell Type</th>
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<td>Skeletal Mb (rat)</td>
<td>42°C, 1 h</td>
<td>↑ survival</td>
<td>HSP72</td>
<td>Suzuki et al., 2000</td>
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<tr>
<td>Skeletal Mb (rat)</td>
<td>42°C, 70 min</td>
<td>↑ survival</td>
<td>HSP70</td>
<td>Maurel et al., 2005</td>
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<td>ESCM (human)</td>
<td>43°C, 30 min</td>
<td>↑ survival</td>
<td>HSP60, HSP70, HSP90</td>
<td>Laflamme et al., 2005</td>
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Table 1. Effect of ischaemic or hypoxic preconditioning on stem and progenitor cells *in vitro*. CLK (cardiosphere-derived Lin<sup>c-kit</sup>+ progenitor cells), CMs (cardiomyocytes), diff. (differentiation), ESCM (embryonic stem cell-derived cardiomyocytes), ESNPCs (embryonic stem cell-derived neural progenitor cells), HO-1 (heme oxygenase-1), HSP (Heat shock protein), KDR (kinase insert domain receptor), Mb (myoblasts), NPCs (neural progenitor cells), NSCs (neural stem cells), PB-MNCs (peripheral blood mononuclear cells), Reoxy (reoxygenation), Wnt4 (wingless-related MMTV integration site 4).

al., 2008; Peterson et al., 2011), erythropoietin (EPO) (Hu et al., 2008; Theus et al., 2008) and NO (Uemura et al., 2006; Li et al., 2005) as the contributing factors. Other potential cytokines and growth factors that have been suggested are basic fibroblast growth factor (bFGF) (Gncechi et al., 2006), hepatocyte growth factor (HGF) (Gncechi et al., 2006), IL-1β (Kubo et al. 2008), IL-6 (Hung et al., 2007a) and thymosin β4 (Gncechi et al., 2006), though more supporting evidence for these factors in mediating the pro-survival effect of HPC in the context of stem cell preconditioning are warranted. In addition, HPC has also been shown to activate several transcription factors and signal transduction cascades that are known to be
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<td>MI (mouse)</td>
<td>MSCs (mouse)</td>
<td>Anoxia, 4 h + 2 h reoxygenation</td>
<td>↓ infarct size &amp; apoptosis; ↑ LV function</td>
<td>Uemura et al., 2006</td>
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<tr>
<td>MI (mouse)</td>
<td>CLK (mouse)</td>
<td>0.1% O&lt;sub&gt;2&lt;/sub&gt;, 6 hours</td>
<td>↓ infarct size; ↑ angiogenesis; ↓ LV remodelling; ↑ LV function</td>
<td>Tang et al., 2009</td>
</tr>
<tr>
<td>MI (rat)</td>
<td>MSCs (mouse)</td>
<td>0.5% O&lt;sub&gt;2&lt;/sub&gt;, 24 h + 2 h reoxygenation</td>
<td>↑ cell survival; ↓ infarct size; ↑ angiogenesis; ↑ LV function</td>
<td>Hu et al., 2008</td>
</tr>
<tr>
<td>MI (rat)</td>
<td>MSCs (rat)</td>
<td>Anoxia &amp; serum-free, 3 h</td>
<td>↓ infarct size &amp; apoptosis; ↑ LV function</td>
<td>He et al., 2009</td>
</tr>
<tr>
<td>MI (rat)</td>
<td>MSCs (rat)</td>
<td>10/30 min anoxia-reoxygenation</td>
<td>↑ cell survival</td>
<td>Kim et al., 2009</td>
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<td>Limb ischaemia (mouse)</td>
<td>MSCs (mouse)</td>
<td>1% O&lt;sub&gt;2&lt;/sub&gt;, 36 h</td>
<td>↑ cell survival; ↑ skeletal muscle regeneration; ↑ limb perfusion; ↑ neovascularization</td>
<td>Leroux et al., 2010</td>
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<tr>
<td>Limb ischaemia (mouse)</td>
<td>MSCs (human)</td>
<td>1-3% O&lt;sub&gt;2&lt;/sub&gt;, 16 h</td>
<td>↑ limb perfusion</td>
<td>Rosova et al., 2008</td>
</tr>
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<td>Limb ischaemia (mouse)</td>
<td>PB-MNCs (mouse)</td>
<td>2% O&lt;sub&gt;2&lt;/sub&gt;, 24 h</td>
<td>↑ cell survival; ↑ limb perfusion; ↑ neovascularization</td>
<td>Kubo et al., 2008</td>
</tr>
<tr>
<td>Limb ischaemia (rat)</td>
<td>MSCs (rat)</td>
<td>2% O&lt;sub&gt;2&lt;/sub&gt;, 24 h</td>
<td>↑ limb perfusion; ↑ neovascularization</td>
<td>Li et al., 2002</td>
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<tr>
<td>Limb ischaemia (rat)</td>
<td>PB-MNCs (human)</td>
<td>pO&lt;sub&gt;2&lt;/sub&gt; of 35mmHg, 7 d</td>
<td>↑ limb perfusion; ↑ neovascularization</td>
<td>Akita et al., 2003</td>
</tr>
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<td>Brain ischaemia (rat)</td>
<td>ESNPCs (mouse)</td>
<td>1% O&lt;sub&gt;2&lt;/sub&gt;, 8 h</td>
<td>↑ cell survival; ↑ recovery of sensorimotor function</td>
<td>Theus et al., 2008</td>
</tr>
<tr>
<td>Diabetic cardiomyopathy (rat)</td>
<td>MSCs (rat)</td>
<td>Anoxia</td>
<td>↓ apoptosis; ↑ angiogenesis; ↓ LV remodelling; ↑ LV function</td>
<td>Li et al., 2008</td>
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<td>Spinal cord injury (rat)</td>
<td>ASCs (human)</td>
<td>1% O&lt;sub&gt;2&lt;/sub&gt;, 24 h + 24 h reoxygenation</td>
<td>↑ survival of co-transplanted NSCs</td>
<td>Oh et al., 2010</td>
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Table 2. Therapeutic potential of hypoxic-preconditioned stem and progenitor cells.

protective and functionally beneficial including the survival kinase Akt (Hung et al., 2007a; Kim et al., 2009), ERK1/2 (Wang et al., 2008a), p38MAPK and survivin (Peterson et al., 2011), SDF-1/CXCR4 and CXCR7 chemokine signalling pathway (Liu et al., 2010), microRNA(miR)-210 (Kim et al., 2009), transcription factors HIF-1α (Kim et al., 2009; Francis & Wei, 2010) and NFκB (Hu et al., 2008). It is also important to note that these mechanistic
pathways and paracrine factors interact with each other and are not mutually exclusive. For example, stabilisation of HIF-1α by HPC, possibly through activation of the PI3K/Akt pathway (Francis & Wei, 2010; Liu et al., 2010), allows its translocation into the nucleus to up-regulate the transcription and translation of various anti-apoptotic proteins such as CXCR7 (Liu et al., 2010), Bcl-2 (Francis & Wei, 2010) and miR-210 (Kim et al., 2009) in stem and progenitor cells. Furthermore, up-regulation of miR-210 has been demonstrated to down-regulate the expression of CAP8AP2, a pro-apoptotic protein that activates death-effector caspase-8 and promotes Fas-induced apoptosis (Kim et al., 2009).

### 3.2 Differentiation and engraftment

In addition to cell survival and retention, lack of significant functional cell engraftment of transplanted cells in the injured tissues has posed another significant challenge for cell-based therapy. In most studies, transplanted stem and progenitor cells do not appear to be trans-differentiated and incorporated into host tissues. Instead, the functional improvement of the ischaemic conditions is likely attributed to the paracrine activities of transplanted cells. Therefore, interventions that can promote stem cell differentiation and functional engraftment in the target tissues post-transplantation should deserve much attention. HPC has been shown not only to enhance stem cell survival but also to promote their differentiation and engraftment. Hypoxia is a potent differentiation inducer of stem cells and studies have demonstrated an acceleration of MSC differentiation when cultured under hypoxic conditions (5-8% O₂) compared with that in normoxic culture, possibly through stabilisation of the oxygen sensitive transcription factor HIF-1α (Lennon et al., 2001; Ren et al., 2006). In contrast, a number of studies have indicated that hypoxia strongly inhibits the differentiation capacity of human bone marrow-derived MSCs (Hung et al., 2007b; Potier et al., 2007; Salim et al., 2004; Volkmer et al., 2010) and adipose-derived mesenchymal stem cells (ASCs) (Malladi et al., 2006; Wang et al., 2005), without affecting the cell viability. Similar conflicting results on stem cell differentiation potential were demonstrated by studies on short-term exposure to hypoxia. Studies on mouse (Theus et al., 2008) and human (Francis & Wei, 2010) embryonic stem cells (ESCs) have indicated a favourable effect of HPC in promoting their neuronal differentiation. A recent study by Volkmer et al. has also reported that HPC can restore the osteogenic differentiation capacity of human MSCs which was otherwise compromised under hypoxic conditions (Volkmer et al., 2010). Conversely, Potier et al. showed that short-term exposure of human MSCs to hypoxia (<1% O₂) has a negative impact on their osteogenic differentiation under normal in vitro culture condition (21% O₂) (Potier et al., 2007). Although the reason for this discrepancy remains unclear, the differences in cell type and species, oxygen tension, duration of exposure to hypoxic conditions and culture conditions could be the answers to these contradictory results.

In terms of functional engraftment, a recent in vitro study by Jaderstad and associates has reported an increased in gap-junctional intercellular communication between hypoxic preconditioned neural stem cells and host cells in vitro, a consequence of increased expression of connexin 43 (Jaderstad et al., 2010). In support of this finding was a previous study reporting that HPC of human MSCs enhanced their xenografting efficiency into chick embryos, a model employed to examine the in vivo engraftment and differentiation potential of stem cells (Hung et al., 2007b). In addition, Xie et al. has reported that conditioned medium from rat neonatal cardiomyocytes subjected to 2 hours of hypoxia followed by overnight reoxygenation can induce MSC differentiation into cardiomyocyte lineage as
indicated by an increase in cardiac myosin heavy chain and troponin T expression (Xie et al., 2006). The latter study has also suggested that HPC may induce secretion of various soluble differentiation factors, whose identity remains to be determined and depend on the cell types. However, what remains unknown is whether these effects of HPC in promoting stem cell differentiation and in vivo engraftment can be translated when preconditioned stem cells are transplanted into adult tissues. To this end, early studies on peripheral blood mononuclear cells (Akita et al., 2003; Kubo et al., 2008) and MSCs (Leroux et al., 2010; Li et al., 2002) have indicated that HPC enhances not only their differentiation into endothelial progenitor cells (EPCs) in vitro but also promotes neovascularisation when transplanted into the ischaemic hindlimb. This effect was associated with improvement of blood perfusion and acceleration of tissue repair. Nevertheless, the lack of detailed histological analysis of angiogenesis in the host tissues, i.e. quantifying the blood vessels derived from implanted cells, has cast doubt on the enhanced functional integration of these transplanted cells as the contributing mechanism (Akita et al., 2003). Instead, the improvement in overall neovascularisation in these studies can be interpreted as a result of increased angiogenic cytokines released by preconditioned cells, such as VEGF (Akita et al., 2003; Leroux et al., 2010; Li et al., 2002), thus promoting intrinsic angiogenesis in the host. Supporting this notion is a study that showed MSCs subjected to ex vivo HPC expressed a higher level of VEGF mRNA and induced greater local VEGF production in the ischaemic hindlimb after implantation, possibly through activation of the canonical Wnt (wingless-related MMTV integration site) pathway (Li et al., 2002; Leroux et al., 2010).

3.3 Cell migration
In cell-based therapy, effective treatment also relies on the ability of transplanted stem and progenitor cells to migrate to the site of injured tissues to exert reparative and regenerative effects. Short-term exposure to hypoxia has been shown to enhance the migratory capacity of stem and progenitor cells ex vivo by modulating the expression of various chemokines and cytokines receptors. Hung et al. reported an upregulation of CXC3R1 and CXCR4 expression on both mRNA and protein levels when MSCs were cultured under hypoxic condition compared with normoxia, resulted in an increased cell migration in response to the fractalkine/CX3CL1 and SDF-1α/CXCL12, respectively (Hung et al., 2007b). A recent study also showed that HPC enhances MSC adhesion, an important step during cell trafficking in vivo, through upregulation of CXCR4 and CXCR7 (Liu et al., 2010). The induction of these chemokine receptors has been shown to be driven mainly by transcription factor HIF-1α (Liu et al., 2010; Hung et al., 2007b; Tang et al., 2009). Using low dose of H2O2 as a preconditioning stimulus, Li et al. also showed that the enhanced chemotaxis of preconditioned MSCs was attributed to the up-regulation of CXCR4 in an ERK-dependent manner (Li et al., 2009). Translating these in vitro findings into an in vivo setting, Tang et al. has shown that short-term exposure of murine cardiac progenitor cells to hypoxia not only enhanced their migratory activity in vitro but also in vivo recruitment to the ischaemic myocardium when administered intravenously, through a CXCR4-dependent manner (Tang et al., 2009). In addition to the chemokine mechanisms, other studies have suggested that HPC enhanced migratory function of stem and progenitor cells through regulation of cytokine signalling (Akita et al., 2003; Rosova et al., 2008; Li et al., 2005). For instance, hypoxia enhanced the migratory function of human EPCs in response to VEGF, possibly through up-regulation of KDR/VEGFR2 expression (Akita et al., 2003). In another study,
Rosova and colleagues showed that HPC increased the expression of the tyrosine kinase receptor, c-Met, in preconditioned MSCs rendered the cells more responsive to HGF (Rosova et al., 2008). Interestingly, HPC can also induce the secretion of chemo-attractants from preconditioned MSCs to promote endothelial cell migration through Wnt4-dependent signalling pathway (Leroux et al., 2010).

### 3.4 Cell proliferation

Stem and progenitor cells self-renew and this is one of the properties that make them an attractive autologous cell source for cell-based therapy and tissue engineering, where success is highly dependent on abundant cell supply.\textit{Ex vivo} cell expansion is traditionally performed under ambient oxygen concentration of 20\% O\textsubscript{2}, which is considered to be hyperoxia compared to their physiological niches (2-7\% O\textsubscript{2}). Thus, it is imperative to simulate various aspects of the stem and progenitor cells’ endogenous microenvironment, including hypoxia, in order to maintain their native characteristics and to comprehend how they respond to a hypoxic environment in injured ischaemic tissues. Studies investigating the effect of hypoxia on stem cell proliferation potential have yielded contradictory results, possibly due to the differences in hypoxic conditions, cell type, serum concentration and culture duration (Das et al., 2010). Compared to the routine normoxic culture of 20\% O\textsubscript{2}, long-term culture of human MSCs in 1\% O\textsubscript{2} has been shown to reduce their proliferative potential (Hung et al., 2007b). Conversely, bone marrow-derived MSCs (D’Ippolito et al., 2006; Grayson et al., 2007; Lennon et al., 2001; Ren et al., 2006), but not ASCs (Wang et al., 2005), cultured under hypoxic conditions with slightly higher oxygen tension (≥2\% O\textsubscript{2}), showed increased cell proliferation. Importantly, short-term exposure to hypoxia did not negatively affect the proliferative potential of stem cells (Francis & Wei, 2010; Leroux et al., 2010; Rosova et al., 2008), an observation that will alleviate the safety concerns of HPC when clinical applications are being considered.

### 4. \textit{Ex vivo} pharmacological preconditioning of stem cells

While ischaemic or hypoxic preconditioning has been shown to regulate multiple stress-responsive mechanisms that promote stem and progenitor cell survival under various ischaemic conditions, preconditioning with specific pharmacological agents seems to target a more linear signalling pathway. This has been explored in various studies on stem and progenitor cells (Table 3).

#### 4.1 Diazoxide

The mitochondrial ATP-sensitive potassium (mitoK\textsubscript{ATP}) channel is an important mediator of cardioprotection (Yellon & Downey, 2003; O’Rourke, 2004) where opening of the channels has been shown to induce protection by preventing calcium overload, inhibiting mitochondrial permeability transition pore (mPTP) opening, preserving ATP production, uncoupling of mitochondrial oxidative phosphorylation, succinate dehydrogenase inhibition, reducing detrimental reactive oxygen species (ROS) production at reperfusion and PKC activation. Using the mitoK\textsubscript{ATP} opener diazoxide, Baines and colleagues were the first to demonstrate that opening of mitoK\textsubscript{ATP} channels prior to ischaemia could mimic the infarct-limiting effect of IPC in the setting of myocardial ischaemia-reperfusion injury (Baines et al., 1999). Recently, diazoxide has also featured in a number of studies by Ashraf’s group to precondition stem and progenitor cells. They have shown that \textit{ex vivo} preconditioning with diazoxide can promote skeletal myoblasts (Haider et al., 2010; Niagara
<table>
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</thead>
<tbody>
<tr>
<td>Diazoxide (200 μM), 30 min</td>
<td>Skeletal Mb (rat)</td>
<td>↑ survival</td>
<td>(MI) ↑ survival; ↑ angiomyogenesis; ↑ LV function</td>
<td>Akt; bFGF; HGF</td>
<td>Niagara et al., 2007</td>
</tr>
<tr>
<td>Diazoxide (200 μM), 30 min</td>
<td>Skeletal Mb (rat)</td>
<td>↑ survival</td>
<td>(MI) ↑ survival &amp; proliferation; ↑ angiomyogenesis; ↑ LV function</td>
<td>Akt; ERK1/2; STAT3; IL-11; miR-21</td>
<td>Haider et al., 2010</td>
</tr>
<tr>
<td>Diazoxide (200 μM), 30 min</td>
<td>MSCs (rat)</td>
<td>↑ survival</td>
<td>(MI) ↑ survival; ↓ infarct size; ↑ angiomyogenesis; ↑ LV function</td>
<td>Akt; Csk3β; NFκB; HGF; IGF; FGF-2; Ang-2</td>
<td>Afzal et al., 2010</td>
</tr>
<tr>
<td>Diazoxide (200 μM), 1-3 h</td>
<td>MSCs (rat)</td>
<td>↑ survival</td>
<td>-</td>
<td>NFκB; Fas; miR-146a</td>
<td>Suzuki et al., 2010</td>
</tr>
<tr>
<td>Diazoxide (200 μM), 30 min</td>
<td>MSCs (rat)</td>
<td>↑ survival</td>
<td>(MI) ↑ survival; ↓ infarct size; ↑ LV function</td>
<td>Akt; bFGF; HGF</td>
<td>Cui et al., 2010</td>
</tr>
<tr>
<td>SDF-1 (50 ng/mL), 1 h</td>
<td>MSCs (rat)</td>
<td>↑ survival; ↑ proliferation</td>
<td>(MI) ↑ survival; ↓ infarct size; ↑ angiomyogenesis; ↑ LV function</td>
<td>Akt; CXCR4; VEGF</td>
<td>Pasha et al., 2008</td>
</tr>
<tr>
<td>SDF-1 (10-100 ng/mL), 24 h</td>
<td>MSCs (rat)</td>
<td>↑ survival</td>
<td>-</td>
<td>CXCR4</td>
<td>Chen et al., 2009</td>
</tr>
<tr>
<td>IGF-1 (100 nM), 30 min</td>
<td>BM Sca-1+ (mouse)</td>
<td>↑ survival; ↑ myogenic diff.</td>
<td>(MI) ↑ survival; ↓ infarct size; ↑ myogenic diff.; ↑ angiogenesis; ↑ LV function</td>
<td>Akt; Cx43</td>
<td>Lu et al., 2009</td>
</tr>
<tr>
<td>IGF-1 (100 nM), 30 min</td>
<td>BM Sca-1+ (mouse)</td>
<td>↑ survival</td>
<td>-</td>
<td>ERK1/2; Cx43</td>
<td>Lu et al., 2010</td>
</tr>
<tr>
<td>TGFα (0.25 μg/mL), 24 h</td>
<td>MSCs (mouse)</td>
<td>↑ apoptosis</td>
<td>(MI) ↓ apoptosis; ↑ LV function</td>
<td>VEGF; p38MAPK</td>
<td>Herrmann et al., 2010b</td>
</tr>
<tr>
<td>TGFα (0.25-1 μg/mL), 24 h</td>
<td>MSCs (human)</td>
<td>↑ VEGF production</td>
<td>-</td>
<td>PI3K; MEK</td>
<td>Wang et al., 2008b</td>
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<tr>
<td>TGFα (0.01-1 μg/mL), 24 h</td>
<td>MSCs (human)</td>
<td>↑ HGF production</td>
<td>-</td>
<td>PI3K; MEK; TNFR; p38MAPK</td>
<td>Wang et al., 2009b</td>
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<tr>
<td>PDGF (0.4 nM), 5 d</td>
<td>MSCs (rat)</td>
<td>↑ adipogenic diff.</td>
<td>-</td>
<td>-</td>
<td>Tamama et al., 2006</td>
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<tr>
<td>PDGF (0.4 nM), 5 d</td>
<td>MSCs (human)</td>
<td>↑ osteogenic- diff.</td>
<td>-</td>
<td>-</td>
<td>Tamama et al., 2006</td>
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<tr>
<td>BMP-2 + bFGF + IGF-1 (10, 50 &amp; 2 ng/mL), 1-7 d</td>
<td>MSCs (rat)</td>
<td>↑ survival; ↑ myogenic diff.</td>
<td>(MI) ↑ survival; ↓ infarct size; ↑ LV function</td>
<td>Cx43; Akt; CREB</td>
<td>Hahn et al., 2008</td>
</tr>
<tr>
<td>IGF-1 + bFGF (50 &amp; 50 ng/mL), 1 h</td>
<td>MSCs (mouse)</td>
<td>↑ survival &amp; proliferation; ↑ angiogenic potential</td>
<td>-</td>
<td>Akt; SOD; Ang-1; Bax; Bak; 16βNκA; p66hsc; p53</td>
<td>Khan et al., 2011</td>
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</table>
### Table 3. Pharmacological preconditioning of stem and progenitor cells.

<table>
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<th>Mechanisms</th>
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</thead>
<tbody>
<tr>
<td>Sevoflurane (2%), 30 min x 3</td>
<td>EPCs (human)</td>
<td>↑ CFC</td>
<td>-</td>
<td>VEGF</td>
<td>Lucchinetti et al., 2009</td>
</tr>
<tr>
<td>Isoflurane (0.5 mM), 10 min</td>
<td>ESCM (human)</td>
<td>↑ survival</td>
<td>-</td>
<td>mitoK&lt;sub&gt;ATP&lt;/sub&gt;; ΔΨ&lt;sub&gt;m&lt;/sub&gt;; ROS</td>
<td>Sepac et al., 2010</td>
</tr>
<tr>
<td>CsA (0.5-5 μM), 30 min</td>
<td>MSCs (rat)</td>
<td>↑ survival</td>
<td>-</td>
<td>Bcl-2; BAD; ΔΨ&lt;sub&gt;m&lt;/sub&gt;</td>
<td>Wang et al., 2008a</td>
</tr>
<tr>
<td>LPS (1 μg/mL), 12 h</td>
<td>MSCs (mouse)</td>
<td>↑ survival</td>
<td>-</td>
<td>TRL-4; Akt; NFκB</td>
<td>Wang et al., 2009c</td>
</tr>
<tr>
<td>LPS (1 μg/mL), 2 d</td>
<td>MSCs (mouse)</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Yao et al., 2009</td>
</tr>
<tr>
<td>Melatonin (5 μM), 24 h</td>
<td>MSCs (rat)</td>
<td>↑ survival; ↑ cell migration; ↑ angiogenic potential</td>
<td>(MI) ↑ survival; ↓ cardiac fibrosis; ↑ angiogenesis; ↑ LV function</td>
<td>Catalase; SOD; bFGF; HGF</td>
<td>Mias et al., 2008</td>
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<tr>
<td>Trimetazidine (10 μM), 6 h</td>
<td>MSCs (rat)</td>
<td>↑ survival</td>
<td>(MI) ↓ infarct size; ↑ LV function</td>
<td>Akt; HIF-1α; survivin; Bcl-2</td>
<td>Wisel et al., 2009</td>
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<tr>
<td>Fucoidan (10 μg/mL), 36 h</td>
<td>EPCs (human)</td>
<td>↑ cell migration; ↑ angiogenic potential</td>
<td>-</td>
<td>-</td>
<td>Zemani et al., 2005</td>
</tr>
<tr>
<td>LPA (10 μM), 1 h</td>
<td>MSCs (rat)</td>
<td>-</td>
<td>-</td>
<td>VEGF</td>
<td>Liu et al., 2009</td>
</tr>
<tr>
<td>Lithium Chloride (5-20 mM), 24 h</td>
<td>Skeletal Mb (rat)</td>
<td>↑ survival &amp; proliferation; ↑ gap junction formation</td>
<td>-</td>
<td>Cx43; VEGF; β-catenin; GSK-3β</td>
<td>Du et al., 2009</td>
</tr>
<tr>
<td>rhHsp90α (0.1-10 μM), 24 h</td>
<td>MSCs (rat)</td>
<td>↑ survival</td>
<td>-</td>
<td>Akt; ERK1/2; NO; Bcl-2; Bcl-xL; Bax</td>
<td>Gao et al., 2010</td>
</tr>
<tr>
<td>β-met (2 mM), 1 h</td>
<td>MSCs (rat)</td>
<td>↑ survival</td>
<td>-</td>
<td>HSP72</td>
<td>Cizkova et al., 2006</td>
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<tr>
<td>rhEPO (10 U/mL), 24 h</td>
<td>ESNPCs (mouse)</td>
<td>↑ survival</td>
<td>-</td>
<td>Bcl-2</td>
<td>Theus et al., 2008</td>
</tr>
<tr>
<td>Carbamylated EPO (100 ng/mL), 30 min</td>
<td>ESCM (human)</td>
<td>-</td>
<td>(MI) ↑ survival</td>
<td>Akt</td>
<td>Robey et al., 2008</td>
</tr>
<tr>
<td>Simvastatin (25 μM), 24 h</td>
<td>EPCs (human)</td>
<td>↑ survival</td>
<td>-</td>
<td>-</td>
<td>Henrich et al., 2007</td>
</tr>
</tbody>
</table>

Ang (angiopoietin), β-met (β-mercaptoethanol), CFC (colony forming capacity), CREB (cAMP response element binding protein), Mb (myoblasts), rh (recombinant human), ΔΨ<sub>m</sub> (mitochondrial membrane potential).
et al., 2007) and MSCs (Afzal et al., 2010; Suzuki et al., 2010) survival both in vitro and in vivo post-transplantation. Furthermore, transplantation of these preconditioned cells into the infarcted myocardium was associated with smaller infarct size, improved LV function, myogenic differentiation and angiogenesis (Afzal et al., 2010; Haider et al., 2010; Niagara et al., 2007). The potential mechanisms responsible for the cytoprotective effect of diazoxide in skeletal myoblasts include enhanced release of paracrine growth factors such as bFGF and HGF, and activation of survival kinase PI3K/Akt (Niagara et al., 2007). A later study by the same group expanded the mechanistic finding to include IL-11, the ERK1/2-STAT3 signalling pathway and up-regulation of miR-21 (Haider et al., 2010). In MSCs, Afzal et al. reported NFκB activation as another important underlying mechanism of diazoxide-induced protection especially during the late phase preconditioning (Afzal et al., 2010). The activation of NFκB in diazoxide preconditioned MSCs was subsequently implicated to regulate the expression of miR-146a, which in turn acts as a negative regulator of the Fas gene, a death receptor of apoptosis (Suzuki et al., 2010).

4.2 Stromal cell-derived factor-1 (CXCR12)

SDF-1 or CXCL12 is a cytokine belonging to the CXC chemokine subfamily. Specific binding of SDF-1 to CXCR4 induces dimerization of the receptor and activates multiple signalling pathways to regulate trafficking and differentiation of stem and progenitor cells (Chen et al., 2011; Kucia et al., 2004). In addition, activation of the SDF-1/CXCR4 axis has been shown to promote cell survival and proliferation (Broxmeyer et al., 2003; Kucia et al., 2004; Hu et al., 2007). The therapeutic potential of SDF-1 was later illustrated in an experimental model of myocardial infarction (Takahashi, 2010). For example, SDF-1 administration conferred cardioprotection through the PI3K/Akt signalling pathway in the setting of acute ischaemia-reperfusion injury (Hu et al., 2007) and chronic ischaemic heart failure (Saxena et al., 2008). In the setting of hindlimb ischaemia, intramuscular injection of SDF-1 increased angiogenic factor VEGF expression, and enhanced the retention and neovascularisation efficacy of transplanted EPCs (Yamaguchi et al., 2003). Other delivery methods for SDF-1 include direct gene delivery with adenoviral vector (Abbott et al., 2004) and plasmid DNA (Hiasa et al., 2004) encoding for SDF-1, or transplantation of genetically modified cardiac fibroblasts (Askari et al., 2003), MSCs (Zhang et al., 2007) or skeletal myoblasts (Elmadbouh et al., 2007) that over-expressed SDF-1 into the ischaemic tissues. Therefore, it is not surprising that SDF-1 has been exploited as a potential preconditioning agent to enhance stem and progenitor cells survival and function without the long-term concern of genetic manipulation. In this regard, pre-treatment with SDF-1 has been shown to enhance MSC survival both in vitro (Chen et al., 2009; Pasha et al., 2008) and following intramyocardial transplantation in the infarcted myocardium through activation of PI3K/Akt signalling pathway and is dependent on CXCR4 (Pasha et al., 2008). In this study, the authors also demonstrated that SDF-1 preconditioning enhances paracrine activities of transplanted MSCs in the infarcted myocardium and contributes to a smaller infarct size, improved cardiac function and promoted revascularization. Interestingly, the cytoprotective effect of SDF-1 appears not to be limited to pre-treatment. Treatment of EPCs with SDF-1 at the end of lethal serum deprivation also significantly reduced apoptotic cell death (Yamaguchi et al., 2003). However, in vivo application of SDF-1 requires vigilant safety evaluation before translating into the clinical setting because of the potentially detrimental side effects of its cleavage.
products. For instance, SDF-1(5-68), a toxic product of SDF-1 cleavage by exopeptidases and metalloproteinase-2, has been implicated in neuroinflammation and neuronal death (Zhang et al., 2003). In which case, SSDF-1(S4V), a modified SDF-1 that is resistant to endogenous proteases cleavage, may have a better clinical safety profile (Segers et al., 2007).

4.3 Growth factors

Various growth factors have been employed to precondition stem and progenitor cells in order to enhance their paracrine activity, cell survival and differentiation (Abarbanell et al., 2009). For example, Lu and colleagues showed that preconditioning with IGF-1 improved bone marrow Sca-1+ stem cell survival against simulated ischaemia in vitro and after in vivo transplantation. This pro-survival effect of IGF-1 was shown to be dependent on the activation of PI3K/Akt (Lu et al., 2009) and ERK1/2 (Lu et al., 2010) signalling pathways, and a downstream mediator connexin-43. A subsequent study indicated that pre-treatment with IGF-1 also enhances Sca-1+ cell cardiomyogenesis potential and transplantation of these preconditioned cells into infarcted heart mitigated myocardial infarction and ventricular dysfunction (Lu et al., 2009). Moreover, MSCs exposed to IGF-1 had an enhanced migratory response to SDF-1, a response dependent on the PI3K/Akt signalling pathway (Li et al., 2007).

It has been well documented that epidermal growth factor (EGF), the prototypical growth factor with intrinsic protein tyrosine kinase activity, plays an important role in the regulation of cell growth, proliferation and differentiation by binding to its receptor (Wells, 1999). In MSCs, EGF has been shown to promote cell proliferation and motility which are beneficial for ex vivo cell expansion prior to in vivo transplantation, but fail to rescue MSCs from low serum-induced apoptosis despite an elevation of Akt. Similar effects were observed with platelet-derived growth factor (PDGF) (Tamama et al., 2006). On the other hand, pre-treatment with TGFα, another member of the EGF superfamily and a potent activator of EGF receptor, has been shown to enhance the therapeutic potential of MSCs in an experimental model of acute myocardial ischaemia-reperfusion injury in terms of myocardial function recovery and inflammation (Herrmann et al., 2010b). These beneficial effects of TGFα have been attributed to the reduction in pro-inflammatory cytokine production and increased VEGF production. The latter effect was shown to be mediated by MEK and PI3K signalling pathways (Herrmann et al., 2010b). It is interesting to note that the effect of TGFα on VEGF production in MSCs has been demonstrated as bimodal, i.e. production of VEGF was suppressed by low concentrations of TGFα whereas high concentrations increased the secretion (Wang et al., 2008b). In addition to VEGF, TGFα treatment also increases the production of HGF from MSCs (Wang et al., 2009b). HGF is an important signalling factor in stem cell-mediated repair where it promotes stem cell adhesion, migration and survival (Vandervelde et al., 2005). Although the potential of HGF as a preconditioning agent to promote stem and progenitor cell survival is yet to be confirmed, existing studies have showed that short-term exposure of MSCs to HGF can activate Akt and ERK1/2 pro-survival kinases, and induce expression of anti-apoptotic protein, Bcl-2 and Bcl-xL (Forte et al., 2006). Additionally, HGF treatment also increased MSC motility function through up-regulation of c-Met receptors and induced their differentiation along a myogenic lineage, effects that can enhance their therapeutic potential in cardiac repair (Forte et al., 2006; Neuss et al., 2004; Rosova et al., 2008). Regarding cell proliferation, HGF treatment was reported to have a negative impact on MSC proliferation by blocking cells in the G0-G1 phase through p38 MAPK pathway and concomitant up-regulation of cell cycle progression inhibitors, p21wafl and p21kip (Neuss et al., 2004; Forte et al., 2006).
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Although different growth factors may share some common downstream signalling pathways in modulating stem and progenitor cell functions, they also independently activate distinct signalling cascades (Abarbanell et al., 2009). This rationale that a combination of multiple growth factors may have additive and synergistic effects in promoting stem and progenitor cell survival and functional improvement, offers a strategic advantage over the approach involving the use of a single factor. In this respect, a recent study by Hahn et al has demonstrated the pro-survival effect of multiple growth factors in MSCs using a combination of IGF-1, bFGF and BMP-2 (Hahn et al., 2008). Treatment with this growth factor cocktail also enhanced MSC cardiac differentiation efficiency and incurred cytoprotection of co-cultured adult cardiomyocytes. This effect was dependent on gap-junction communication leading to phosphorylation of Akt and c-AMP response element binding protein (CREB) in cardiomyocytes. Moreover, the therapeutic efficacy of MSCs in infarcted myocardium was also significantly potentiated with this multiple growth factors preconditioning method (Hahn et al., 2008). Similarly, Khan et al preconditioned MSCs isolated from diabetic mice with a combination of IGF-1 and bFGF and showed enhanced cell survival, proliferation, motility and angiogenic potential compared to untreated cells or cells treated with single growth factor (Khan et al., 2011). Furthermore, a potent synergistic effect between TGFβ and TNFα has been demonstrated to enhance the MSC-derived VEGF and HGF production that may be essential for cell survival, migration and angiogenesis (Herrmann et al., 2010a; Wang et al., 2008b; Wang et al., 2009b).

4.4 Anaesthetics
Anaesthetics, routinely administered in patients during surgery, are widely recognised to have preconditioning properties protecting the heart and many other organs against ischaemia and reperfusion injury in laboratory settings as well as in humans with coronary heart diseases (Huffmyer & Raphael, 2009; Yellon & Downey, 2003). The efficacy of anaesthetic-induced preconditioning has been shown to be similar to IPC in terms of infarct size reduction, increased collateral blood flow and attenuated inflammatory responses during ischaemia. Although the mechanisms underlying the protective effect of anaesthetic-induced preconditioning have been shown to resemble those responsible for IPC (Zaugg et al., 2003), Mullenheim et al showed that administration of sevoflurane combined with IPC can synergistically protect rabbit hearts against myocardial infarction indicating the existence of parallel protective mechanisms (Mullenheim et al., 2003). In ESC-derived cardiomyocytes, brief exposure to the commonly used volatile anaesthetic, isoflurane, significantly attenuated cell death against oxidative stress through opening of mitoKATP channels, production of signalling ROS and inhibition of mPTP opening (Sepac et al., 2010). Other benefits include enhanced cell growth capacity and increased expression of pro-angiogenic VEGF as demonstrated in human EPCs preconditioned with sevoflurane (Lucchinetti et al., 2009).

4.5 Cyclosporin-A (CsA)
The mPTP is a non-specific channel of the inner mitochondrial membrane, whose opening at the onset of reperfusion is a critical mediator of lethal myocardial ischaemia-reperfusion injury. IPC exerts its cardioprotective effect by inhibiting the opening of the mPTP and pharmacological inhibition of mPTP with CsA has been shown to confer cytoprotection both in vitro and in vivo (Hausenloy & Yellon, 2003; Lim et al., 2007). In addition to inhibiting mPTP opening, CsA can also inhibit calcineurin-mediated dephosphorylation of the apoptotic
protein BAD and uncouple the mitochondrial respiratory chain, which might, in itself, result in protection (Wilkins et al., 2004). Interestingly, mPTP can also open reversibly under basal conditions without causing cell death. This form of reversible non-pathological mPTP opening has been noted to contribute to the cardioprotection elicited by IPC through the mitochondrial generation of signalling ROS and the subsequent activation of the pro-survival kinases Akt and ERK1/2 (Hausenloy et al., 2010). In stem cells, pre-treatment with CsA has been shown to protect MSCs from hypoxia-reoxygenation induced apoptosis through stabilizing mitochondrial membrane potential and promoting Bcl-2 and phosphorylated BAD protein expression (Wang et al., 2008a). In line with this study, we recently showed that pre-treatment with 0.2 μM of CsA for 30 minutes effectively increased the resistance of human ASCs to subsequent simulated ischaemia-induced cell death (Figure 2, unpublished data).

![Fig. 2](image.png)

**Fig. 2.** Preconditioning with CsA confers cytoprotection in stem cells. Pre-treatment with CsA (0.2 μM, 30 min) reduced cell death (determined by propidium iodide staining, arrow heads) in hASCs subjected to 15 hours of simulated ischaemia (<1% O2 and ischaemic buffer as described previously (Lim et al., 2008)) from 58.5±2.6% to 42.5±1.8% (n=4-5, P < 0.05).

### 4.6 Lipopolysaccharide
LPS is an antigenic component of the outer membrane of gram-negative bacteria and an agonist of Toll-like receptor-4 (TLR4) capable of eliciting immune responses in animals. The cardioprotective effect of LPS preconditioning was previously demonstrated in an in vivo murine model of myocardial ischaemia-reperfusion injury (Ha et al., 2008). Evidence supporting the pro-survival effect of LPS preconditioning in MSCs was recently provided by Wang and colleagues. The authors reported that activation of Toll-like receptor-4 (TLR4) with low dose of LPS can prevent MSCs from apoptotic cell death induced by oxidative stress and serum deprivation through PI3K/Akt- and NFκB-dependent mechanisms (Wang et al., 2009c). Furthermore, transplantation of these LPS-preconditioned MSCs into infarcted rat hearts significantly improved cardiac function, reduced apoptosis and fibrosis, and enhanced angiogenesis (Yao et al., 2009).

### 4.7 Other agents
Other drugs and hormones have also been employed as preconditioning agents to promote stem and progenitor cell survival and function. For example, pre-treatment with the pineal hormone melatonin can improve the therapeutic effectiveness of MSCs in the setting of acute renal ischemia-reperfusion injury by potentiating their survival and paracrine activity.
(Mias et al., 2008). **Trimetazidine** is a cytoprotective anti-ischaemic agent that acts to reduce ischaemia-induced metabolic damage by shifting the energy substrate preference from fatty acid oxidation to glucose oxidation. It has been employed recently to precondition MSCs and showed increased cell survival and enhanced therapeutic potential in reducing myocardial ischaemic injury (Wisel et al., 2009). **Fucoidan**, a high molecular weight sulphated polysaccharide, also favourably enhanced the migratory potential of pre-treated EPCs *in vitro*. Although alone it did not promote angiogenesis, treatment with fucoidan potentiated the angiogenenic effect of bFGF in EPCs (Zemani et al., 2005). Du et al. preconditioned skeletal myoblasts with **lithium chloride** and showed enhanced cell survival and increased gap-junctional coupling with co-cultured neonatal cardiomyocytes, a result of increased Cx43 expression (Du et al., 2009). Similarly, pre-treatment with the endogenous phospholipid signalling molecule, **lysophosphatidic acid (LPA)**, can improve MSC survival *in vivo* in ischaemic myocardium and enhance their angiogenic effects (Liu et al., 2009). Interestingly, an *in vitro* study by the same group indicated that LPA failed to precondition MSCs (Chen et al., 2008). In this study, the anti-apoptotic effect of LPA was only evident when the drug was present throughout the hypoxia and serum deprivation insult but not when LPA was removed after the pre-treatment period. Nevertheless, the cytoproteective effect of LPA was demonstrated to be dependent on the activation of LPA receptor-1, and pertussis toxin-sensitive PI3K/Akt and ERK pathways (Chen et al., 2008).

**Heat shock proteins** (HSP) are known protective mediators of preconditioning induced by ischaemia, hypoxia, heat stress and oxidative stress (Das & Maulik, 2006) (Table 1). In MSCs, preconditioning with recombinant human HSP90α produced an anti-apoptotic effect via activation of PI3K/Akt and ERK signalling pathways (Gao et al., 2010). Prior to this study, genetic modification to over-express HSP20 had been shown to enhance the survival of MSCs against oxidative stress and improve their therapeutic potential in ischaemic rat heart (Wang et al., 2009a). Therefore, it is not surprising that drugs capable of increasing HSP expression are potential preconditioning agents. Indeed, short-term exposure to **β-mercaptoethanol** has been shown to protect MSCs from oxidative injury, a cytoprotective effect associated with an elevation of HSP72 expression (Cizkova et al., 2006).

The haematopoietic cytokine **erythropoietin** (EPO) also exerts cardioprotection in both animal and clinical studies with many intracellular signalling pathways implicated, including PI3K/Akt, ERK1/2, p38 MAPK, PKC, eNOS and guanylyl cyclase and the opening of mitochondrial K<sub>ATP</sub> channels (Riksen et al., 2008). In MSCs (Hu et al., 2008; Theus et al., 2008) and embryonic stem cell-derived neural progenitor cells (NPCs) (Francis & Wei, 2010; Theus et al., 2008), EPO is already involved in the cytoprotective effect of HPC. As a preconditioning agent, recombinant human EPO mimicked the cytoprotective effect of HPC in protecting mouse NPCs against serum deprivation-induced apoptotic cell death (Theus et al., 2008). Similarly, ESC-derived cardiomyocytes survived better in the infarcted mouse hearts when they were preconditioned with carbamylated EPO prior to implantation (Robey et al., 2008). The hydroxyl-methylglutaryl coenzyme A reductase inhibitors, also known as statins, are effective for lowering serum cholesterol and have been widely prescribed for patients with coronary heart diseases as the primary and secondary preventive treatment of cardiovascular events. In addition to lipid-lowering effects, statins have been shown to exert multiple pleiotropic effects including protection from MI, improved endothelial function and reduced platelet adhesion and atherosclerotic plaque rupture (Ludman et al., 2009). In MSCs, treatment with **lovastatin** has been shown to improve cell survival when challenged
with hypoxia and serum deprivation, a protection mediated by PI3K/Akt and ERK1/2 pathways (Xu et al., 2008). Similarly, human EPCs pre-treated with simvastatin also exhibit higher resistance against TNFα induced apoptotic cell death (Henrich et al., 2007).

5. Ischaemic or hypoxic preconditioning in vivo mobilises endogenous stem and progenitor cells

Endogenous stem and progenitor cells can be mobilized from their niches in various organs and tissues, including bone marrow, skeletal muscle, heart, brain, skin, liver, adipose, blood vessels and others, and then home to target tissues. This property of stem and progenitor cells has fuelled investigations of potential clinically adaptable strategies to actively recruit endogenous stem and progenitor cells to serve as integrated participants in regenerating the injured tissues through stem cell trans-differentiation and/or as supportive players via pleiotropic paracrine effects (Chen et al., 2011; Krankel et al., 2011). By subjecting rats to 3 weeks of chronic hypoxia, Rochefort et al have unveiled the potential of HPC in mobilising endogenous MSCs. In this study, circulating MSCs were higher in rats subjected to chronic hypoxia compared to the control normoxic cohort (Rochefort et al., 2006) (Table 4). Interestingly, this hypoxic condition did not affect the circulating level of haematopoietic stem cells (HSCs) indicating a possible cell-type specific effect of HPC (Rochefort et al., 2006). In line with this study, rats preconditioned with 6 hours of hypoxia daily for 6 weeks also have a higher level of CD34+CXCR4+ cells in their blood circulation and in the infarcted hearts with concomitant reduction in acute myocardial ischaemia-reperfusion injury (Lin et al., 2008). Using a relatively more invasive preconditioning protocol of transient coronary artery occlusion and reperfusion, Ii and colleagues have showed that IPC modulates endogenous EPC kinetic and increases their recruitment to the infarcted myocardium (Ii et al., 2005). This observation was associated with infarct size limitation, increased angiogenesis and cardiac function improvement, beneficial effects that were shown to be strongly dependent on the iNOS and eNOS activities of the EPCs (Li et al., 2005). The mobilizing and homing effect of IPC was subsequently illustrated on other cell types such as MSCs and HSCs using a clinically relevant porcine myocardial ischaemia-reperfusion injury experimental model (Gyongyosi et al., 2010). Excitingly, Kamota et al showed that preconditioning applied on the abdominal aorta can also increase the accumulation of bone marrow-derived sca-1+ and c-kit+ stem cells in infarcted hearts through a SDF-1/CXCR4-dependent mechanism, thus protecting the hearts against injury (Kamota et al., 2009). This finding is clinically important as it supported the translation of the non-invasive strategy of remote IPC into clinical practise. Remote IPC is a clinically amenable strategy which can be induced by simple transient limb ischaemia. This cardioprotective strategy has been extensively trialled in patients undergoing cardiac surgeries such as coronary angioplasty and coronary artery bypass surgery, and has thus far showed tangible beneficial effects in reducing myocardial injury with no known adverse risks (Hausenloy & Yellon, 2008).

Although the precise molecular and cellular mechanisms governing the homing effect of ischaemic/hypoxic preconditioning on endogenous stem and progenitor cells remain to be fully addressed, evidence of a role for the chemokine axis SDF-1/CXCR4 (Kamota et al., 2009; Lin et al., 2008) and EPO (Lin et al., 2008) has been found in recent studies. Other possible stem cell homing factors include VEGF, colony-stimulating factor, monocyte
<table>
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<tr>
<th>PC stimulus</th>
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<tr>
<td>Hypoxia; 24 h of 50kPa (O_2) daily for 3 weeks</td>
<td>Rat, Sham</td>
<td>↑ circulating MSCs</td>
<td>-</td>
<td>Rochefort et al., 2006</td>
</tr>
<tr>
<td>Hypoxia; 6 h of 10% (O_2) daily for 6 weeks</td>
<td>Rat, MI</td>
<td>↑ circulating &amp; heart CD43+CXCR4+ cells; ↓ infarct size; ↑ LV function; ↓ plasma CK-MB</td>
<td>SDF-1/CXCR4; EPO; VEGF</td>
<td>Lin et al., 2008</td>
</tr>
<tr>
<td>Ischaemia; 4 x 4 min/4 min coronary artery O/R</td>
<td>Mouse, MI</td>
<td>↑ circulating &amp; heart EPCs; ↓ infarct size; ↑ LV function; ↑ angiogenesis</td>
<td>eNOS; iNOS; VEGF</td>
<td>Li et al., 2005</td>
</tr>
<tr>
<td>Ischaemia; 2 x 5 min/5 min coronary artery O/R</td>
<td>Pig, MI</td>
<td>↑ circulating HSCs; ↑ heart MSCs &amp; HSCs; ↓ infarct size &amp; apoptosis; ↑ LV function</td>
<td>SDF-1α; VEGF; TNFα; IL-8</td>
<td>Gyongyosi et al., 2010</td>
</tr>
<tr>
<td>Ischaemia; 4 x 5 min/5 min abdominal artery O/R</td>
<td>Mouse, MI</td>
<td>↑ circulating CD43+flk-1+ cells; ↑ heart Sca-1+ &amp; c-kit+ BMSCs; ↓ infarct size &amp; apoptosis; ↑ LV function</td>
<td>SDF-1/CXCR4; VEGF</td>
<td>Kamota et al., 2009</td>
</tr>
<tr>
<td>Ischaemia; 25 min/7 d renal artery O/R</td>
<td>Mouse, RI</td>
<td>↑ renal EPCs</td>
<td>-</td>
<td>Patschan et al., 2006</td>
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Table 4. Effect of in vivo ischaemic/hypoxic preconditioning on endogenous stem and progenitor cells. BMSCs (bone marrow stem cells), O/R (occlusion/reperfusion), RI (renal ischaemia).

chemotactic protein-3, HGF, IGF-1, IL-8/growth regulated oncogene-1, stem cell factor, TGF-\(\beta\)3, Wnt antagonist and other chemokines (Krankel et al., 2011; Binger et al., 2009; Chen et al., 2011). However, whether these navigational factors govern the homing effect of \textit{in vivo} IPC warrants further investigation. In perspective, a living body appears to host a great reservoir of various stem and progenitor cells ready to be recruited for regeneration and repair, and can be catalysed by external stimuli such as IPC. This is of great clinical importance because \textit{in vivo} IPC can be readily and non-invasively achieved in patients. However, given the fact that majority of ischaemic disease sufferers are elderly patients, and endogenous stem and progenitor populations and functions are known to decline with age and are negatively affected by other co-morbidity such as diabetes and hypertension (Krankel et al., 2011), it is important to determine whether there are sufficient populations of functionally competent resident stem and progenitor cells to be mobilised and recruited to the target tissues to exert significant repair in older patient cohorts. Otherwise, a combination of exogenously administered cells and potent homing factors might be utilised to supplement the endogenous reservoirs for effective cell-based therapy. Therefore, detailed characterisation of various endogenous stem cell niches and deciphering the mechanisms governing endogenous stem cell repopulation, mobilisation and homing to target tissues are pivotal to the future development of clinically sound pharmacological interventions to harness fully the host’s innate regenerative capacity.
6. Conclusion

Based on the existing literature, it is undeniable that non-genetic approaches of preconditioning techniques offer much promise as cytoprotective strategies for stem cell therapy, without the long-term concern of genetic manipulation. These pro-survival strategies are therefore well suited to clinical translation. Collectively, preconditioning of stem and progenitor cells elicits multiple beneficial effects including (1) promotion of cell survival in the hostile ischaemic environment, (2) enhancement of paracrine activity to create a supportive environment that is rich in trophic and angiogenic factors, (3) increase of cell motility and trafficking, (4) increase of cell proliferative potential, (5) promotion of cell differentiation allowing functional integration, and (6) enhancement of therapeutic efficacy in ischaemic tissues \textit{in vivo}.

In cell based therapy for ischaemic diseases, the ability of preconditioning \textit{ex vivo} to enhance stem and progenitor cell survival and function means more implanted cells will be available for tissue repair and thus fewer donor cells may be needed to achieve the same functional outcome. In parallel, \textit{in vivo} preconditioning is capable of harnessing the host’s inherent regeneration mechanisms through activation of various paracrine signalling cascades, and mobilizing and recruiting resident stem and progenitor cells for effective therapeutics (Figure 3). Therefore, it will be of great therapeutic interest to determine whether there is an additive or synergistic effect of \textit{ex vivo} preconditioning of implanted cells and \textit{in vivo} preconditioning of host tissues, which may provide an optimal regenerative environment for tissue repair and regeneration, and contribute to successful stem cell therapy and tissue engineering.

![Diagram of Preconditioning](https://www.intechopen.com)

Fig. 3. Potential additive effect of \textit{ex vivo} preconditioning (transplanted exogenous stem cells) and \textit{in vivo} preconditioning (patients) to enhance tissue repair and tissue engineering with stem cells.
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Even if the origins of regenerative medicine can be found in Greek mythology, as attested by the story of Prometheus, the Greek god whose immortal liver was feasted on day after day by Zeus' eagle; many challenges persist in order to successfully regenerate lost cells, tissues or organs and rebuild all connections and functions. In this book, we will cover a few aspects of regenerative medicine highlighting major advances and remaining challenges in cellular therapy and tissue/organ engineering.

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